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FORMULATION DEVELOPMENT AND EVALUATION OF IN SITU GEL OF LEVOFLOXACIN AND DEXAMETHASONE

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ABSTRACT

Poor bioavailability (<10%) of drugs from conventional eye drops is mainly due to the various precorneal loss factors which include rapid tear turnover, systemic drug absorption through naso-lachrymal duct, transient residence time of the drug solution in the cul-de-sac and the relative impermeability of the drugs to corneal epithelial membrane. In situ gels are systems which are applied as solutions or suspensions and are capable of undergoing rapid sol-to-gel transformation triggered by external stimulus such as temperature, pH etc on instillation. The purpose of the present research work was to develop and evaluation sustains release *in situ* ophthalmic gel of dexamethasone (DXM) and levofloxacin (LEV). The different gelling solutions were made with Pluronic F127 in combination with different polymers such as Carbopol 934 and HPMC, which acted as a viscosity-enhancing agent. Suitable concentrations of buffering agent were used for pH adjustment. All the formulations were sterilized in an autoclave at 121°C for 15 minutes. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content, rheological study, sterility testing, in vitro drug release study, ocular irritation study and stability study. The developed formulations exhibited sustained release of drug from formulation over a period of 7 hours. The prepared formulations were tested for eye irritation on albino rabbit (male). The results demonstrated that the developed *in situ* gel of DXM and LEV is nonirritant, has prolonged action and is a better option in terms of retention, ocular bioavailability and patient compliance when compared with plain eye drops formulation.

K**eywords:** Dexamethasone, Levofloxacin, In situ gel, In vitro drug release, Rheological study.

INTRODUCTION:

Eye is one of the challenging organs for drug delivery because of its unique anatomy restricts drug absorption into deeper tissues [1]. Major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to precorneal loss resulting in only a small fraction of the drug being ocularly absorbed. The effective dose administered may be altered by increasing the retention time of medication into the eye by using in situ gel-forming systems. Ophthalmic drug delivery is an extremely interesting and highly challenging endeavor [2,3]. The anatomy, physiology, and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge

to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage [4]. Ophthalmic ointments ensure superior drug bioavailability by increasing the contact time, minimizing the dilution by tears, and resisting nasolacrimal drainage. Major disadvantage of ointment, providing blurred vision, due to this it could be used either night time or for treatment on the outside and edges of the eyelids. Suspension as ophthalmic delivery systems relies on the assumption that particles may persist in conjunctival sac. Precorneal drug loss can be minimal, such as retarding drainage by using diffusion-controlled, non-erodible polymeric insert. The major disadvantage of inserts is the lack of patient acceptance owing to difficult administration. The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of more successful ocular delivery systems. The primitive ophthalmic solution, suspension, and ointment dosage forms are clearly no longer sufficient to combat these diseases, and current research and development efforts to design better therapeutic systems are the primary focus of this research work. A thermoresponsive in situ gel, an ophthalmic product vehicle responding to a shift in temperature, possesses liquid characteristic at room temperature and becomes gel when comes in contact with body temperature. One of well-known polymer types possessing thermoresponsive behaviour is Pluronics, so called Poloxamers. They are a triblock copolymer poly (ethylene oxide)-bpoly(propylene oxide)- b-poly(ethylene oxide) (PEO–PPO–PEO) showing amphiphilic behavior due to hydrophilic ethylene oxide domains and hydrophobic propylene oxide domains. The gelation mechanism of Pluronics could be explained by the changes in micellar structure as a function of concentration and temperature [5-7]. However, a major disadvantage of Pluronics is their low mucoadhesive activity, therefore, some Pluronic based ophthalmic formulations have been improved by adding polymers providing mucoadhesive property such as cellulose derivatives. LEV, Biopharmaceutical Classification System I, is a broad spectrum anti-infective agent, under the third generation fluoroquinolone derivative mainly used in the infection of the eye such as acute conjunctivitis. The recommended dosage of LEV for the treatment of bacterial conjunctivitis is 1 or 2 drops of 0.5% solution in the affected eyes for every 2 hours upto 8 times for 2 days, then 1 or 2 drops every 4 hours up to 4 times for next 5 days [8]. LEV is quickly and fully absorbed subsequent to oral administration. Peak plasma concentrations are typically attained one to two hours subsequent to oral dose. The normal terminative plasma elimination half-life of levofloxacin is ranging from around 6 to 8 hours consequent to single or multiple doses of levofloxacin administered either intravenously or orally [9]. Dexamethasone (figure 1) is a synthetic adrenocortical steroid and is chemically designated as 9-fluoro-11β, 17, 21-trihydroxy-16α-methylpregna-1, 4-diene, 3, 20-dione. The drug is 25 times more potent than cortisol in its glucocorticoid effect, while having minimal mineralocorticoid effect. It has anti-inflammatory and immunosuppressant effects and is used for the treatment of many conditions including rheumatologic

problems, a number of skin diseases such as erythema multiforme, severe allergies, asthma, chronic obstructive lung disease, croup, cerebral edema, in addition to other medications in tuberculosis and a number of other infectious diseases. However, it is characterized by a low water solubility (0.08 mg/ml at 25°C) and consequently low and irregular bioavailability [10]. Also the therapeutic efficacy has been questioned with regard to its short half-life [11], potential toxicity at high doses [12]. The present study aimed to prepare and evaluate ophthalmic in-situ gel formulations of DXM and LEV. In situ gel solution increases the residence time and also sustain the release mechanism of the drug.

MATERIALS AND METHODS

Materials

Levofloxacin (LEV) was obtained as a gift sample from Micro Labs Ltd., Bangalore, India. Dexamethasone (DXM) was obtained as gift samples from MSN labs, Hyderabad. PluronicF127 was obtained from Sigma Aldrich, Mumbai. Benzalkonium chloride from Merck Ltd, Mumbai, Sodium chloride from Loba chemicals, Hydroxypropyl methylcellulose (HPMC-15cps) and carbopol-934 from Central Drug House, Mumbai, India. All other chemicals and solvents were of analytical grade and used as received. Distilled water was prepared in laboratory using all glass distillation apparatus.

Methods

Determination of λ_{max} of LEV and DXM

Accurately weighed 10 mg of drugs was dissolved in 10 ml of methanol in 10 ml of volumetric flask separately. The resulted solution 1000µg/ml and from this solution 0.1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.2 pH phosphate buffer solution prepare suitable dilution to make it to a concentration of 10µg/ml for LEV and DXM. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+).

Formulation development

Selection of vehicle

The solubility of LEV and DXM was tested in various buffers such as acetate buffer I.P. (pH 6.0 and 6.5), citrophosphate buffer B.P. (pH 6.0 and 6.2) and phosphate buffer USP (pH 7.2 and 7.4) in order to select a suitable vehicle. Solutions of LEV and DXM in the above buffers were prepared to test its solubility at the dosage level desired.

Preparation of in-situ gel

For the preparation of Pluronic F127 based ocular in-situ gel all the ingredients were sieved from sieve no 44. Solution of 0.5% and 0.1% of drugs was prepared in acetate buffer I.P. pH 5.0. The solution was cooled in an ice bath and pluronic F127 was added slowly with continuous stirring. Then the resulting solution was kept in a refrigerator under 4^0C for 24h. This storage helped in dissolving the pluronic F127

completely. After 24h carbopol 934 and HPMC 15cps were added slowly along with other excipients with continuous stirring for 2-3 hours for proper mixing and avoiding slug formation. Buffering and osmolality agents were added to the resulting solution along with benzalkonium chloride. The pH of the solution was adjusted using 0.5 N NaOH. The resulting formulation was kept on probe sonicator to remove air bubble. All formulations were stored in LDPE (Low Density Polyethylene) bottles for further use [13]. All the containers were stored in refrigerator. Composition of different formulations of in-situ gel is given in Table 1

Table 1 Composition of different formulations of *In-situ* gel

S. No.	Ingredient (%)	Formulations								
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
1.	Levofloxacin	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
2.	Dexamethasone	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
2.	Pluronic F127	18	16	14	18	16	14	18	16	14
3.	Carbopol 934	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
4.	HPMC 15cps	1.0	1.0	1.0	0.75	0.75	0.75	0.5	0.5	0.5
5.	EDTA	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
6.	Benzalkonium Chloride	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%	0.013%
7.	NaCl	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
8.	PEG	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
9.	Acetate Buffer (pH 5.0)	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Evaluation of in situ gelling system

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

Drug content

The assay of drug LEV and DXM was performed by UV method. The calculation was based on calibration curve method using regression equation (Y=mx+c).

pH

The pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using calibrated digital pH meter.

In-Situ gelling capacity

In situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37^0C. Formulations were introduce into STF in a ratio of 1:2 Change in consistency of formulations were visually inspected.

Viscosity study

At pH 5.0 and temperature less than 16^0C, the developed formulations were in liquid state and show low viscosity. For viscosity studies the pH of formulations were raised from pH 5.0 to pH 7.4 and the temperature was raised to 37^0C. The pH was raised to 7.4 by the addition of 0.5M NaOH. The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 RPM for comparative study. The angular viscosity was measured by gradually increase the RPM from 10 to 70.

Sterility testing

Sterility

The test for sterility is applied to pharmacopoeial articles that are required according to the Pharmacopoeia to be sterile. However, a satisfactory result only indicates that no contaminating viable micro-organisms have been found in the sample examined in the conditions of the test. The test must be carried out under aseptic conditions designed to avoid accidental contamination of the product during testing. For achieving these conditions, a grade a laminar airflow cabinet or an isolator is recommended. The test environment has to be adapted to the way in which the tests are performed. Precautions taken for this purpose should not adversely affect any micro-organisms, which are to be revealed in the tests. The working conditions in which the tests are carried out should be monitored regularly by appropriate sampling of the air and surfaces of the working area and by carrying out control tests.

Culture media

The following culture media have been found to be suitable for the test. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it will also detect aerobic bacteria. Soyabean-casein digest medium is suitable or the culture of both fungi and aerobic bacteria.

Method of test

For aqueous solutions. Remove the liquid from the test containers with a sterile pipette or with a sterile syringe or a needle. Transfer the quantity of the preparation under examination into the culture medium so that the volume of the preparation under examination is not more than 10 per cent of the volume of the medium, unless otherwise prescribed. If the preparation under examination has antimicrobial activity, carry out the test after neutralising this with a suitable neutralizing substance or by dilution in a sufficient quantity of culture medium. When it is necessary to use a large volume of the product it may

be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution. Where appropriate, the concentrated medium may be added directly to the product in its container. Incubate the inoculated media for not less than 14 days. Observe the cultures several times during the incubation period. Observe the containers of media periodically during the 14 days of incubation. If the test specimen is positive before 14 days of incubation, further incubation is not necessary. For products terminally sterilized by a validated moist heat process, incubate the test specimen for not less than 7 days.

In-vitro drug release study

The *in vitro* release of drugs from the formulations was studied through cellophane membrane. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots (each of 1ml) were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium [14-16].

Results and discussion

The λ_{max} of LEV and DEX was found to be 288 and 244 nm respectively by using U.V. spectrophotometer (Labindia-3000+) in linearity range 5-25 µg/ml Fig.1 & 2. Formulations were evaluated for clarity by visual observation against a black and white background. Some formulations had problem of precipitation of carbopol during storage, the problem overcome by increasing the stirring time up to 2-3 hours during formulation. Only those formulations were selected for further studies which were clear. The drug content of both the drug in formulations was determined by UV method. The developed formulations were evaluated for pH by using digital pH meter. The pH of formulations was decreases from buffer pH 5.0 because of acidic groups of carbopol so that the pH was adjusted to 5.0 by using 0.5N NaOH. *In situ* gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37^0C. Solution was introduced into STF in a ratio of 1:2 Changes in consistency of solution visually inspected. Formulation F3, F6 and F9 show poor gelling capacity in simulated physiological conditions of pH and temperature because of comparatively less concentration of pluronic F127 in IG6 and F9 and due to low concentration of carbopol in F1. F4 and F5 formulation show better gelling capacity Table 2. Viscosity of formulation was determined before and after gelation by using Brookfield's viscometer in the small volume adaptor and the angular velocity was increased gradually

from 10, 20, 40, 50, 60 and 70 RPM. The comparative study of viscosity was done at 50 RPM. F4, F5, and F7 show comparatively better viscosity and good consistency gel Table 3. All the formulations terminally sterilized by autoclaving. The process was executed by placing the formulations in borosilicate conical flask these flasks these flasks are placed in a preheated autoclave. Then the autoclave well closed by clamps. Formulations were sterile by heating at 121^0C for 15 minutes under pressure. For sterility testing formulations were diluted ten times by sterile distilled water. From this dilution remove quantity and placed in culture media, this quantity should be equivalent to more than 200 mg of the formulation. Petri dishes then placed in incubation chamber for 7 days and observed for microbial growth Table 3. The percentage cumulative drug release of the prepared formulations showed sustained drug release up to 7 hours duration. Formulation F5 showed more sustained release compared to other formulations. The percent cumulative drug release (%CDR) of F5 was given in Table 4 & 5. The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic equsation, Higuchi's and Korsmeyer's models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of first order was maximum hence indicating drug release from formulations was found to follow first order release kinetics Table 6 and Fig3-6 .

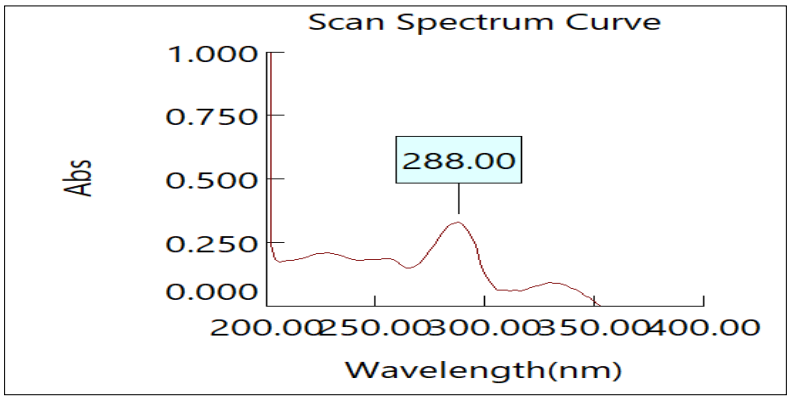

Figure 1 Determination of λ_{max} of Levofloxacin

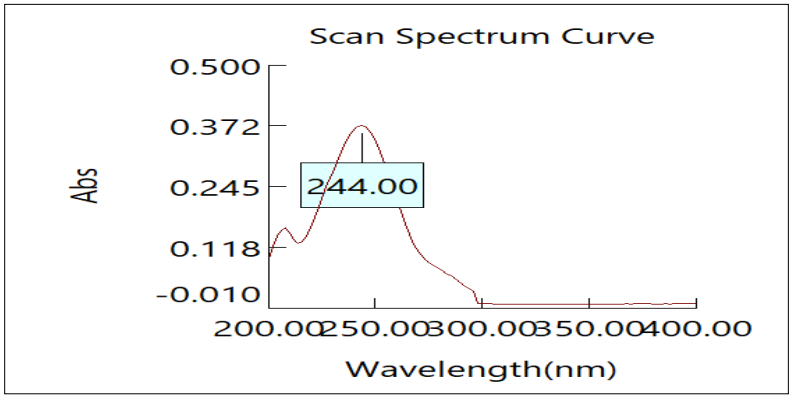

Figure 2 Determination of λ_{max} of Dexamethasone

Table 2 Physical parameters of gel formulations

F. code	Clarity	pH	*In situ* gelling capacity	Drug Content (%)*	
				LEV	DXM
F1	Clear	4.1	"++"	98.22	96.65
F2	Clear	4.3	"++"	99.14	99.89
F3	Turbid	4.2	"+"	97.22	98.56
F4	Clear	3.9	"+++"	98.65	97.85
F5	Clear	3.8	"+++"	95.51	97.65
F6	Clear	4.1	"+"	95.56	98.12
F7	Precipitate observed	3.9	"++"	96.69	95.65
F8	Clear	4.0	"++"	97.89	98.85
F9	Clear	4.2	"+"	98.25	98.78

*****Average of three determinations (n=3)
"+" gelation after five minutes and dissolves rapidly
"++" gelation immediate, remains for few hours
"+++" gelation immediate, remains for extended period 8 hours

Table 3 Comparative viscosity* of *In situ* formulation and sterility testing

F. code	% of Pluronic F 127	Viscosity of solution (in cps)	Viscosity after gelation	Observation
F1	18	987	2423	No growth
F2	16	881	2205	No growth
F3	14	811	2150	No growth
F4	18	1053	2671	No growth
F5	16	933	3056	No growth
F6	14	613	2330	No growth
F7	18	741	2685	No growth
F8	16	771	2535	No growth
F9	14	668	2831	No growth

*Spindle no.7, Rpm 50

Table 4 *In Vitro* drug release profile of LEV from *In Situ* formulation F5

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.00	1.301	80.00	1.903
1	1.000	0.000	35.56	1.551	64.44	1.809
1.5	1.225	0.176	40.23	1.605	59.77	1.776
2	1.414	0.301	51.12	1.709	48.88	1.689
3	1.732	0.477	63.32	1.802	36.68	1.564
4	2.000	0.602	69.98	1.845	30.02	1.477
5	2.236	0.699	73.32	1.865	26.68	1.426
7	2.646	0.845	75.65	1.879	24.35	1.386

Table 5 *In Vitro* drug release profile of DEX from *In Situ* formulation F5

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	22.23	1.347	77.77	1.891
1	1.000	0.000	36.65	1.564	63.35	1.802
1.5	1.225	0.176	45.56	1.659	54.44	1.736
2	1.414	0.301	55.56	1.745	44.44	1.648
3	1.732	0.477	68.89	1.838	31.11	1.493
4	2.000	0.602	73.32	1.865	26.68	1.426
5	2.236	0.699	75.56	1.878	24.44	1.388
7	2.646	0.845	78.89	1.897	21.11	1.324

Table 6 Comparative study of regression coefficient for selection of optimize formulation F5

	Zero order	First order
Levofloxacin	$R^2 = 0.827$	$R^2 = 0.908$
Dexamethasone	$R^2 = 0.800$	$R^2 = 0.876$

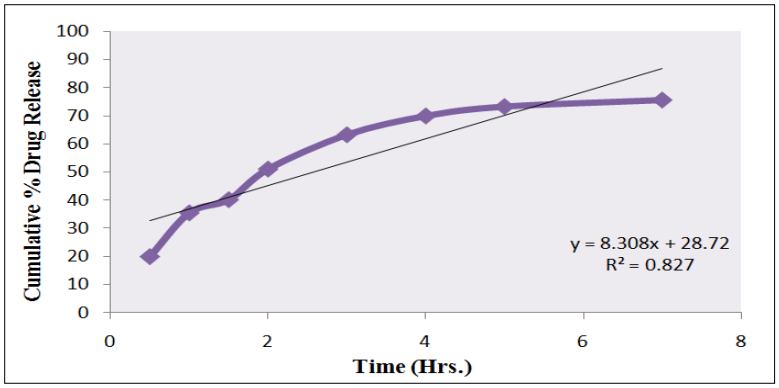

Figure 3 Zero order release Kinetics of LEV

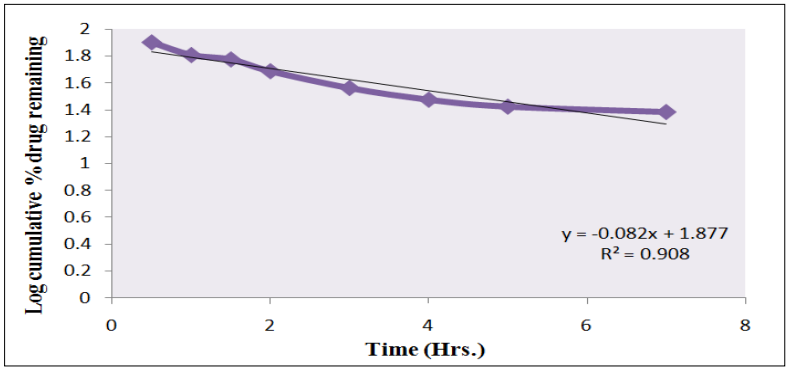

Figure 4 First order release kinetics of LEV

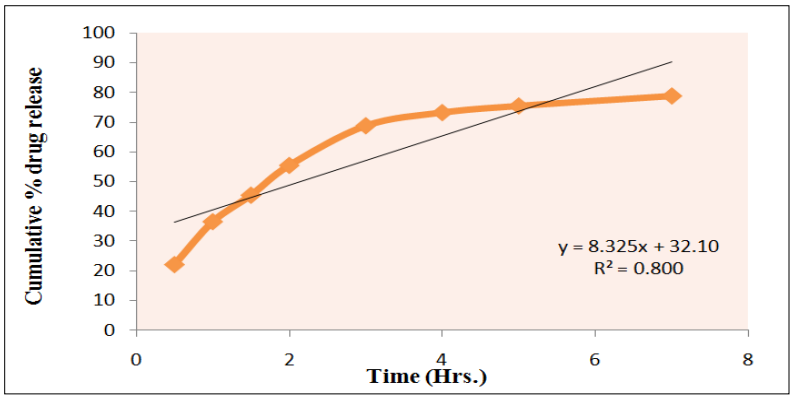

Figure 5 Zero order release kinetics of DEX

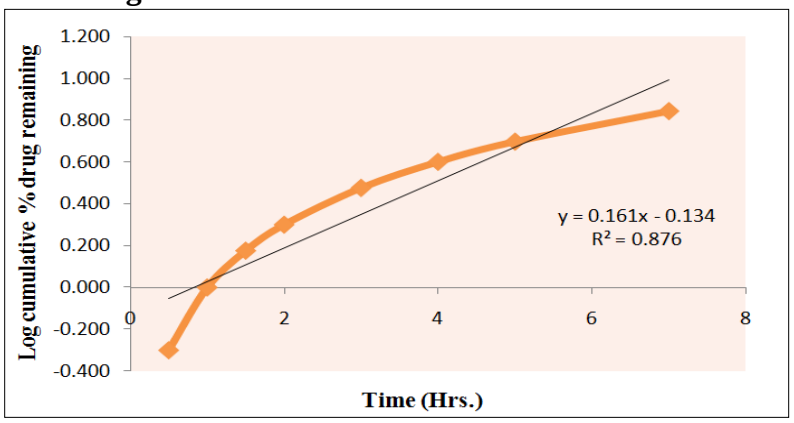

Figure 6 First order release kinetics of DEX

Conclusion

The objective of the present research work was to develop a sustained release ocular drug delivery system with improved patient compliance and longer precorneal resistance time. Based on in vitro and in vivo characterization, we concluded that the developed in situ gelling formulation is a nonirritant, nontoxic sustained release formulation system for sustained topical drug delivery to eyes. This new formulation of LEV and DEX is a viable option for effective and controlled management of conjunctivitis and other eye related disorders. The drug was intact and stable in the in situ gelling formulation during storage.

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